1760-Pos Board B530
A Two-State Eukaryotic Cell Migration Model
Osman N. Yogurtcu, Sean X. Sun.
Johns Hopkins University, Baltimore, MD, USA.
Cells are dynamic and motile objects and the way they make decisions about migratory directions has been an area of intensive research. To explain the intricate effects of genetic mutations and environmental changes on cell migration, we have devised a discrete stochastic trajectory decision model from simple mechanochemical principles. In the current model, the cells have been treated as mobile machines with two states of migration: persistent and random. The state transitions are assumed to obey Markovian dynamics. We used molecular diffusion and polymer analogies to estimate likelihoods of the trajectories. The model has been tested on human fibrosarcoma and fibroblast wild type cells, various knockdowns, and cells performing chemotaxis and galvanotaxis. The results demonstrate the adequacy of the model in fitting the experimental cell speed distribution, velocity autocorrelation, mean squared displacement and angles between consecutive velocity steps. The model has the potential to better explain more complex trajectory dynamics of cells including cancer invasion, wound healing, and cellular development.

1761-Pos Board B531
Distinct Roles for Talin-1 and Kindlin-3 in Regulating LFA-1 Conformation during Neutrophil Rolling and Arrest
Craig T. Lefort[1], Jan Rossaint[2,3], Markus Moser[4], Brian G. Petrich[5], Alexander Zarbock[2,3], Susan J. Monkley[6], David R. Critchley[6], Mark H. Ginsberg[5], Reinhard Fässler[4], Klaus Ley[1].
[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA, [2]University of Münster, Münster, Germany, [3]Max Planck Institute of Molecular Biomedicine, Münster, Germany, [4]Max Planck Institute of Biochemistry, Martinsried, Germany, [5]University of California San Diego, La Jolla, CA, USA, [6]University of Leicester, Leicester, United Kingdom.
β2 integrins, including LFA-1 (αLβ2), play critical roles in leukocyte trafficking. LFA-1 mediates the recruitment of neutrophils during inflammation through interaction with ICAM-1 expressed on the endothelium. The affinity of integrin for ligand is determined by its global conformation, regulated by the binding of proteins to the integrin cytoplasmic tails. At least three different integrin affinity states have been observed: low affinity with bent ectodomain, intermediate affinity with extended ectodomain, and high affinity with extended ectodomain and open headpiece. Intermediate affinity LFA-1 contributes to neutrophil rolling interactions with the endothelium, whereas high affinity LFA-1 mediates neutrophil arrest. In the current study, we determined the roles for two integrin tail-binding activating proteins, talin-1 and kindlin-3, in neutrophil rolling and arrest. Using established *in vivo* (intravital microscopy of post-capillary venules) and *ex vivo* (microfluidic flow chamber) models, we found that talin-1 is necessary for both LFA-1-dependent neutrophil rolling and arrest. In contrast, kindlin-3 is required only for neutrophil arrest and is dispensable for rolling mediated by intermediate affinity LFA-1. These data indicate that talin-1 is sufficient to induce LFA-1 extension to an intermediate affinity state whereas kindlin-3 is critical for the transition of LFA-1 to a high affinity state. We further corroborated our results with imaging and *in vitro* studies using antibodies that directly report β2 integrin conformation. Our results are the first to demonstrate that members of the talin and kindlin protein families play distinct roles in regulating integrin conformation.

1762-Pos Board B532
Force at Early Adhesion Formation: Integrin Activation Triggers Contractile Movement and Subsequent Outward Translocation of Integrin-RGD Clusters
Cheng-han Yu[1], Michael P. Sheetz[1,2].
[1]Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [2]Department of Biological Sciences, Columbia University, New York, NY, USA.
Integrin-mediated adhesions play important roles in many biology events, including stem cell differentiation, cancer metastasis, and the immune response. However, The mechanism of early adhesion formation remains unclear. Using mobile RGD ligands on lipid bilayers with nano-fabricated physical barriers, we observe surprising long-range lateral movements of ligated integrins during the process of cell spreading. At first, RGD-activated integrin clusters stimulate actin polymerization that radiates from the clusters. Myosin II contraction of actin from adjacent clusters produces contractile pairs that move toward each other against barriers. Force generated by myosin II stimulates a Src kinase-dependent lamellipodial extension and outward movement of clusters. Subsequent retraction by myosin II causes inward movement of clusters. The final cell spread area increases with the density of periodic barriers. Early integrin clustering recruits adhesion proteins, talin, paxillin and focal adhesion kinase, irrespective of force generation. However, recruitment of vinculin is only observed upon contraction. Thus, we suggest that integrin activation and early clustering are independent of lateral forces. Src-dependent actin polymerization on integrin clusters and subsequent contraction stimulate active spreading with outward forces from actin polymerization followed by a second wave of contraction. These early mechanical steps provide new targets to control integrin-dependent adhesion and motility.

1763-Pos Board B533
A Universal Molecular Tension on a Single Integrin during Cell Adhesion
Xuefeng Wang, Taekjip Ha.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The integrin is a cell-membrane receptor that mediates cell attachment to the extracelluar matrix (ECM). As mechanosensors, integrins also sense and transduce mechanical information of ECM into the cells to regulate cell morphology and migration. Cellular traction forces applied through an ensemble of integrins have been extensively characterized but the cellular tension on a single integrin that needs to be sustainably applied is unknown. Here we developed a molecular tension sensor to measure the tension at a single integrin during cell adhesion to ECM. The ligand of integrin αVβ3, cyclic RGDfK peptide, is synthesized and immobilized to a polymer-passivated surface through a variety of tethers with various rupture forces ranging from 15 pN to 50 pN. During cell adhesion, integrins bind to RGDfKs and transduce tension to the tether. When the tension exceeds its defined tolerance, the tether is ruptured and the ligand separates away from the surface so that the cell does not adhere. A tension titration test with nine different tethers of differing tension tolerance was performed on CHO-K1 cells. We found that cells only adhere and spread on the surface with a tension tolerance of 30 pN or higher. Other cell lines were also tested including HEK 293, MDA-MB-231, Hela cells and NIH/3T3 cells. Despite the large variations in the bulk traction forces known to exist among these cells, the critical tension tolerance required remained at 30 pN. We therefore propose that cells apply a universal tension across a single integrin-ligand pair during cell adhesion. The underlying molecular mechanisms for the universal molecular tension is under investigation. Our approach is generally applicable to other cellular signaling processes and may reveal the physiologically relevant tension magnitudes across single receptor-ligand pairs using a cell physiology readout.

1764-Pos Board B534
Dependence of Forces and Ahdesion Structures of Primary Myocytes on Substrate Elasticity
Nils Hersch[1], Georg Dreissen[1], Ronald Springer[1], Norbert Kirchgessner[1,2], Bernd Hoffmann[1], **Rudolf Merkel**[1].
[1]Forschungszentrum Juelich, Juelich, Germany, [2]Institute of Agricultural Science, ETH Zurich, Zurich, Switzerland.
Force generation is the main physiological function of primary heart muscle cells (myocytes). To study this experimentally, we cultivated primary myocytes from neonatal rat pups on silicone elastomer substrates of controlled elasticities. Young's moduli ranged from 1 to 500 kPa covering physiological and pathological heart tissue stiffnesses. Fluorescent microbeads embedded in the surface of these substrates served as markers for the displacements caused by spontaneously contracting cells. Forces of isolated cells were extracted from these displacements by the method of traction force microscopy (TFM). Transfection with GFP-α-actinin enabled visualization of cell adhesions simultaneously to TFM. By restricting the force application points chosen for the TFM algorithm to certain adhesion structures their mutual relevance for cellular traction force application was explored. Moreover, total cell forces clearly depended on substrate stiffness. These experiments were complemented by a quantification of myofiber contraction. Here, GFP-α-actinin containing z-bands of separate myofibers were imaged by fluorescence microscopy. Subsequent digital image processing was used to quantify the local contraction of individual fibers on different stiffnesses. In contrast to forces, fiber contraction appeared to depend little on substrate stiffness.

1765-Pos Board B535
Correlating Actin Stress Fibers to Extracellular Forces
Hedde van Hoorn, Dominique Donato, Thomas Schmidt.
Leiden University, Leiden, Netherlands.
Most eukaryotic cells constantly probe the mechanical properties of their environment. During this process the actin cytoskeleton is contracted by myosin II activity. At the same time actin is linked through focal adhesion proteins and integrins to extracellular proteins. Our goal is to gain insight in the mechanical coupling between cytoskeletal contractility and the extracellular forces generated. In our experimental approach we use fluorescence imaging and simultaneous local force-detection using microstructured elastomeric pillars.
Micropillars are prepared from Poly-DiMethylSiloxane (PDMS) through replica molding in a hexagonal pattern with: 2 μm diameter, 4 μm center-to-center

spacing and a height of 4-12 μm. In this range forces of 1-50 nN per pillar are measured. The PDMS pillars were stamped with partly fluorescently labeled fibronectin that allowed us to accurately determine the pillar deflections. Subsequently, 3T3 mouse fibroblasts were seeded onto the pillars. Immunostaining was employed using standard procedures to visualize the actin cytoskeleton and focal adhesion complexes. The actin cytoskeleton, focal adhesions and pillar deflections were imaged with a confocal spinning disk setup. From these results, we quantified the degree of co-orientation of focal adhesion elongation with force direction and the increase in stress fiber- and focal adhesion size with forces in the range of 1-15 nN.

1766-Pos Board B536

Mechanical Strain in Actin Networks Regulates FilGAP and Integrin Binding to Filamin A

Allen J. Ehrlicher[1,2], Fumihiko Nakamura[3], John H. Hartwig[2], David A. Weitz[1], Thomas P. Stossel[2].
[1]Harvard University, Cambridge, MA, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Harvard Medical School, Boston, MA, USA.

Mechanical stresses elicit cellular reactions mediated by chemical signals. Defective responses to forces underlie human medical disorders, such as cardiac failure and pulmonary injury. Despite detailed knowledge of the cytoskeleton's structure, the specific molecular switches that convert mechanical stimuli into chemical signals have remained elusive. Here we identify the actin-binding protein, filamin A (FLNa) as a central mechanotransduction element of the cytoskeleton by using Fluorescence Loss After photo Conversion (FLAC), a novel high-speed alternative to FRAP. We reconstituted a minimal system consisting of actin filaments, FLNa and two FLNa-binding partners: the cytoplasmic tail of ß-integrin, and FilGAP. Integrins form an essential mechanical linkage between extracellular and intracellular environments, with ß integrin tails connecting to the actin cytoskeleton by binding directly to filamin. FilGAP is a FLNa-binding GTPase-activating protein specific for Rac, which *in vivo* regulates cell spreading and bleb formation. We demonstrate that both externally-imposed bulk shear and myosin II driven forces differentially regulate the binding of integrin and FilGAP to FLNa. Consistent with structural predictions, strain increases ß-integrin binding to FLNa, whereas it causes FilGAP to dissociate from FLNa, providing a direct and specific molecular basis for cellular mechanotransduction. These results identify the first molecular mechanotransduction element within the actin cytoskeleton, revealing that mechanical strain of key proteins regulates the binding of signaling molecules.

''Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A''
A.J. Ehrlicher, F. Nakamura, J.H. Hartwig, D.A. Weitz and T.P. Stossel. Nature (2011) doi:10.1038/nature10430.

1767-Pos Board B537

Insights into the Importance of Mechanosensing through the Focal Adhesion Protein p130Cas

Dominique Donato, Hedde van Hoorn, H. Emrah Balcioğlu, Erik Danen, Thomas Schmidt.
Leiden University, Leiden, Netherlands.

The Src substrate p130Cas has previously been identified as one of several focal adhesion proteins implicated in mechanosensing, the process by which cells feel changes in their physical environment. As a protein that has also been implicated in enhanced cell migration, actin dynamics, and focal adhesion turnover, the manner by which p130Cas orchestrates such sensing is becoming of increasing interest. In vitro studies have suggested that the central substrate domain may relay mechanosensing information via spring-like stretching of this domain. However, in vivo detection of such a mechanism has not been possible to date. To gain greater insight into this problem, we used polyacrylamide substrates of varying rigidities and observed the abilities of mouse embryonic fibroblasts (MEFs) to adhere, spread, and form focal adhesions by spinning disk confocal microscopy. By comparing cells lacking and stably re-expressing p130Cas, we addressed the following questions: 1) How sensitive is p130Cas to these mechanical changes (i.e. at what rigidity does p130Cas first become tyrosine phosphorylated)? 2) Is p130Cas critical to the ability of the cell to sense these changes (analyzed by tracking changes in focal adhesion characteristics as well as cell spread)? and 3) What is the mechanism underlying the ability of p130Cas to transduce mechanical signals (i.e. is there a spring-like domain within p130Cas)? Our multi-parameter experimental strategy allowed us to analyze increases in cell spread and focal adhesion organization as substrate rigidity was altered from ~100 Pa to 69 GPa. Interestingly, we observed that MEFs deficient for p130Cas displayed changes in cell spread and focal adhesion rearrangement at lower rigidity scales than was observed in wild-type counterparts, illustrating a need for p130Cas to sensitize cells to substrate rigidity.

1768-Pos Board B538

The Non-Equilibrium Thermodynamics and Kinetics Governing Coupled Stress Fiber and Focal Adhesion Dynamics

Krishnakumar Garikipati, Jianping Fu, Mirko Maraldi.
University of Michigan, Ann Arbor, MI, USA.

We consider the coupled mechano-chemistry governing the cytoskeletal force in cells with stress fibers and focal adhesions. Our studies include published experimental work in which we monitor the cytoskeletal forces in cells on elastomeric substrates of micron-sized posts. These experiments demonstrate complex dynamics involving substrate strain as well as the binding/unbinding of cytoskeletal and focal adhesion proteins. Our model explains these dynamics, which underlie force generation and motility of, especially, mesenchymal cells. The broader motivation for this research comes from our ongoing work on cancer cell motility and invasion. Guided by our experiments, our model considers a single stress fiber, the focal adhesion by which it is attached to a micropost, the cell reservoir of cytoskeletal and focal adhesion proteins, the deforming micropost and underlying substrate. The complex mechano-chemistry that controls these sub-systems' interaction is itself governed by non-equilibrium thermodynamics: The binding/unbinding of proteins is driven by free energy changes due to chemistry, elasticity and mechanical work done. The stress fibers' mechanical response has viscoelastic and active contributions, the latter due to myosin contractility. Our model, an extension of our recently published work, generates a very rich range of responses depending on the mechanical and chemical boundary conditions, and parameter values (which are obtained from our experiments, and well-established estimates from the literature). This range of model responses includes every case observed in our experiments. We find that while applied strain and acto-myosin contractility dictate the increase of force in stress fibers over the short to medium time scale (~600 sec.), the longer time scale response (~1000-10000 sec) is dominated by the growth and disassembly of focal adhesions. These findings have direct implications for published and ongoing work on cancer cell locomotion in our group.

1769-Pos Board B539

Exploring Feedbacks Between Small GTPases, Phosphoinositides, and the Actin Cytoskeleton

Leah Edelstein-Keshet.
University of British Columbia, Vancouver, BC, Canada.

I survey work done in my group on eukaryotic cell polarity and motility. The crosstalk and feedback between small GTPases, phosphoinositides (PIs), and F-actin has been probed both experimentally and computationally. In so doing, we have identified both likely connectivity of these signaling networks and their functional roles in motility. We found that GTPases can account for most essential polarity-motility function, but PIs sensitize the cells, and help to filter out conflicting spatial cues. This work is joint with Stan Maree, William R Holmes, Ben Lin, Andre Levchenko, and Veronica Grieneisen.

1770-Pos Board B540

The Geometry of Branching Actin Networks from Capping Branching and Filament Growth

Daniel B. Smith[1], Jian Liu[2].
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]NIH - NHBLI, Bethesda, MD, USA.

Branching actin network growth is the primary engine driving cell motility. At the front of a motile cell, inside the lamellipodium, is a dense mesh of actin filaments pushing the membrane forward. The structure of the mesh is believed to be largely regulated by three processes: branching of new filaments off of existing ones, capping of filament tips stopping filament growth, and filament growth. Filaments inside the lamellipodium have been observed to organize into a strict orientation pattern where filaments are angled approximately −35/35 degrees from the direction normal to the membrane. It has been previously hypothesized that the three processes above are sufficient to generate the unique orientation pattern.

We derive and analyze an integro-differential PDE for the angular density of branching actin network by incorporating the three constituent processes. Our analysis implies that there exist multiple equilibrium angular distributions, which strongly suggests additional process that regulates actin filament orientation.

1771-Pos Board B541

Unraveling the Role of the SCAR/WAVE Complex in Regulating the Traction Stresses during Amoeboid Motility

Effie Efthimia Bastounis, Ruedi Meili, Baldomero Alonso Latorre, Juan Carlos, del Alamo, Richard Firtel, Juan Lasheras.
UCSD, San Diego, CA, USA.

Chemotaxis, or guided cell migration plays an essential role in many key physiological and disease processes. Chemotaxis requires a tightly regulated,